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Laterality in cavefish: Left or right foraging behavior in *Astyanax mexicanus*

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Abstract

The evolution of foraging behaviors is key to colonizing challenging habitats such as a cave's dark environment. Vibration attraction behavior (VAB) gives fish the ability to swim in the darkness toward a vibration stimulus produced by many prey crustaceans and insects. VAB evolved in the blind Mexican cave tetra, *Astyanax mexicanus*. VAB is regulated by an increased number of mechanosensory neuromasts, particularly in the eye orbital region. However, VAB in *Astyanax* is only correlated with the number of neuromasts on the left side. *Astyanax* also have a bent skull preferentially to the left and a QTL signal for the right-side number of neuromasts. We conducted field studies in five different cave populations for four years. Results support that all cave populations can express behavioral lateralization or preponderance of side to examine a vibrating object. The percentage of individuals favoring one side may change among pools and years. In one cave population (Pachón), for one year, this "handedness" was expressed by preferentially using the right side of their face. On the contrary, in most years and pools, Tinaja, Sabinos, Molino, and Toro cave populations explored preferentially using their left side. This suggests that if there is an adaptative effect, it selects for asymmetry on itself, and not necessarily for which side is the one to be specialized. Results also showed that the laterality varied depending on how responsive an individual fish was, perhaps due to its nutritional, motivational state, or mode of stimuli most relevant at the time for the fish.

Keywords

asymmetry, Sierra de El Abra, Stygobite, Troglobite, Troglomorphy, VAB

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Introduction

The blind Mexican tetra, *Astyanax mexicanus* (=*Astyanax jordani*), is an emerging model system for the study of evolution. Great advances have been made in understanding the genetic basis of adaptation to an extreme environment, such as living in continuous darkness. The freshwater fish from genus *Astyanax* has two morphs: an eyed surface morph and an eyeless cave morph. The cave morph is restricted to 32 known limestone caves in the Sierra de El Abra region of Northeastern Mexico, and two caves in Guerrero, in Southcentral Mexico (Espinasa et al. 2020). The closely related surface morphs are broadly distributed throughout surface streams and lakes in Mexico.

Throughout their evolution, *Astyanax* cave-dwelling fish have undergone regression of their eyes and pigmentation, whereas the performance of the mechanosensory lateral lines, the olfactory, taste buds, chemosensors, and their foraging behavior have been enhanced to cope with the continuous darkness (Wilkens 1988; Yamamoto and Jeffery 2000; Yamamoto et al. 2004, 2009; Jeffery 2005; Protas et al. 2006; Menuet et al. 2007; Gross et al. 2009; Varatharasan et al. 2009; Yoshizawa et al. 2010; Bibliowicz et al. 2013; Keene et al. 2015; Blin et al. 2018).

The swimming of fish toward an oscillating object, or Vibration Attraction Behavior (VAB) is one of the enhanced behaviors shown in cavefish (Parzefall 1983; Abdel-Latif et al. 1990; Yoshizawa et al. 2010). VAB is mediated by an increase in the cranial superficial neuromasts (Yoshizawa et al. 2010). VAB is aroused in cavefish by vibrations of around 35 Hz (Yoshizawa et al. 2010), which is within the main range produced by many potential prey crustaceans and insects (Lang 1980; Montgomery and Macdonald 1987). The advantage of VAB in foraging has been shown in competitive prey capture assays in the dark (Yoshizawa et al. 2010: Espinasa et al. 2014). VAB appears adaptive in cavefish since it increases foraging ability in an environment devoid of light, with sparse food and few macroscopic predators.

Espinasa et al. (2021) showed that VAB may be present in all cave populations throughout the range of hypogean *A. mexicanus*, spanning from Sierra the Guatemala in the north to southern Sierra de El Abra. However, and most notably, within a given cave the levels of VAB are highly variable between different pools. It appears that individuals inhabiting pools with a high abundance of organic matter have reduced expression of VAB, while in pools with little organic debris, where fish probably depend on hunting more than on scavenging, VAB is enhanced (Espinasa et al. 2021).

Surface morphs are largely symmetric across the left-right axis with little variation (Gross et al. 2015). In contrast, cave-adapted fish have evolved extreme cranial asymmetries. This asymmetry is displayed with a "bend" in their skulls along the anteroposterior axis that is most often biased to the left (Powers et al. 2017). An asymmetric body pattern may negatively affect swimming performance. For example, it could be expected that unequal lateral hydrodynamic resistance (drag) may be energetically costly and result in a preponderance for swimming in circles when visual cues are absent. It can be hypothesized that if cavefish have evolved an extra expenditure due to the drag generated by a bent head, there could be an adaptive compensation from such a unique feature.

Some of the main skull asymmetries are found in the dermal bones that encircle the eye orbit and, in particular, on the third suborbital (SO3) bone, which is commonly fragmented in an irregular architecture across the lateral cranial complex (Gross et al. 2014). Tactile sensitivity in cavefish is mediated by the lateral line, a sensory system comprised of mechanosensory neuromasts. The superficial neuromasts distributed within the eye orbit region of the face are the most important in mediating VAB (Yoshizawa et al. 2010). However, further analyses by Fernandes et al. (2018) showed lateralization; cavefish from Pachón cave show a positive correlation of VAB with the number of neuromasts on their left side but not on their right. This laterality was not seen in *Astyanax* surface fish under any conditions tested, suggesting this laterality emerged through an evolutionary process. They concluded that *Astyanax* cavefish have higher developmental plasticity in neuromast number and bone formation and that cavefish showed an asymmetric correlation between the number of left-right neuromasts and VAB.

Subsequent studies have shown that neuromasts placement and suborbital bone formation are linked during development and that canal neuromast position prefigures developmental patterning of the suborbital bone series in *Astyanax* cave- and surface-dwelling fish (Powers et al. 2018). Using a QTL approach, Powers et al. (2020) showed that the genetic architecture of neuromasts present in the eye orbit region is under genetic control with a lateral asymmetry; linked loci signals were detected for the right side of the face, but not for the left side. The locus for neuromast position maps near established QTL for eye size and facial bone morphology, suggesting that eye loss, sensory expansion, and the cranial skeleton may be integrated for evolving adaptive behaviors. It should be pointed out that while there is a QTL signal for the number of neuromasts only on the right side of the face, the positive correlation of VAB is with the number of neuromasts on only their left side. Both studies used lab stocks derived from Pachón cave (QTL specimens were derived from specimens originally collected by Dr. Richard Borowsky of New York University, while the ones for the VAB studies were collected by Dr. William Jeffery of The University of Maryland).

These findings, a bent skull preferentially to the left, a correlation of VAB with left side number of neuromasts (in Pachón cave), and a QTL signal for the right-side number of neuromasts, suggest laterality in enhanced sensitivity for different modes of stimuli and the potential for behavioral laterality with different side swimming preference, or handedness, for different behaviors. While there is no preponderance for swimming direction in familiar settings in the laboratory, cavefish from Pachón cave prefer to use their right-side lateral line when encountering a novel stationary landmark (De Perera and Braithwaite 2005) but until now, it is unknown if there is a side preponderance for any foraging behavior including VAB.

In hypothesizing for an adaptive reason for a curved or bent skull, we first tested if the skull or the body of *A. mexicanus* had a size that could theoretically reflect 30 and 40 Hz waves to amplify a signal, similar to a parabolic amplifier. To work as a parabolic amplifier for waves, the corresponding parabola size must be of the wavelength order or bigger (Rumsey and McCormick 2006). We then tested whether there is laterality

in response to a vibrating stimulus in their natural habitat. Considering that different cave populations may have different evolutionary histories and that the expression of VAB can differ even between pools of the same cave, field research was conducted on 11 different pools in five caves encompassing the Sierra de Guatemala and the Sierra de El Abra, on four different years.

Methods

In the water, potential prey can emit both sound waves and ripple waves. To test if the curved shape of a fish while circling a vibrating stimulus or the bent shape of a cavefish skull have the minimum theoretical size to reflect either type of waves in such a way as to focus wave signals, the following formulas were used. Wavelength λ can be computed if propagation speed υ_{phase} and frequency ν are known: $\lambda = \upsilon\, phase/\, \nu$.

Sound (pressure waves) travels through water at a speed close to 1,480 m/s. Yet, surface ripples travel much slower in deep water (depth > λ). In a previous study (Espinasa et al. 2021), the speed of ripples on the water surface of the actual pools inhabited by cavefish was measured for 35 Hz frequencies, with results between 10.7 and 17.2 cm/s. With these results, a plot was made for wavelengths versus phase velocities to determine the theoretical minimum size of cavefish structures that could serve as amplifiers.

For the field experiments, the care and use of experimental animals complied with Mexican animal welfare laws, guidelines, and policies Fieldwork Mexican permits 02438/16, 05389/17 and 1893/19 (Patricia Ornelas-Garcia and LE) were delivered by the Secretaria de Medio Ambiente y Recursos Naturales. The cave populations studied were Molino, Pachón, Sabinos, Tinaja, and Calera (within the Toro cave system). The first is located in the Sierra de Guatemala and the rest in the Sierra de El Abra, all in Northeast Mexico. For a full description of the caves, their topography, localization, and ecology, see Elliott (2018) and Espinasa et al. (2020).

An apparatus for delivering vibrations was obtained and modified to meet the requirements for waterproofness, portability, and reliability needed for the trying conditions of fieldwork in a cave. A vibrator device connected to a potentiometer to control the amount of current and thus speed control was used (Espinasa et al. 2021). Calibration and corroboration of the vibration frequency emitted by the apparatus were done as in Espinasa et al. (2021). The average value of the actual frequencies that were delivered in the field in multiple pools was 35.5 Hertz, with a standard deviation of 6.29, a minimum frequency of 31.5, and a maximum frequency of 42.6. The video analyses started only a minimum of 5 sec after the device entered the water to reduce the effect of the sound of the device first entering the water. The process was repeated alternatively with the vibrator on and off. Results for the difference between on and off can be found in Espinasa et al. (2021); when vibration is on, fish swim towards the device, and then highly responsive individuals spin in circles around the device, oftentimes biting it.

Video recordings were done in February of 2022 of two pools from Pachón cave and one pool from Tinaja cave. Pachón's "Large pool" is the one first encountered when entering the cave and 31 recording were performed there. 150 m further in, there is the "Maryland pool" in the Maryland extension portion of the cave and 56 recordings were performed here. Tinaja's "4th pool" is found deep into the cave in the section dubbed "Sandy Floored", and 21 recording were done. When the first fish circled once around the vibrating device, filming was stopped and the device was taken out of the pool. The individual fish that conducted the behavior was captured with a hand net and deposited in an inflatable pool. This guaranteed that data for statistical analyses corresponded to single events conducted by different fish within the population. Vibrating device was not put back into the water until about 5 minutes later. Filming was performed with a DCR-SR42 Sony Digital camera. VLC Media Player was used to analyze the collected videos. The expected proportion of fish performing left-side turns versus right-side under the null hypothesis is 0.5. Statistical significance was determined using the binomial test (The non-parametric X^2 test with a Yates correction for continuity [Yates 1934], as recommended when $\upsilon = 1$ [Zar 1984] gave equivalent and corroborating P values).

We were also interested in analyzing behavior in other caves and across multiple years. For this, video clips produced by Espinasa et al. (2021) were used, giving data for comparisons among five different caves and 11 pools over four different years. When those videos were taken, the purpose of the study was simply to determine the presence or absence of VAB. Therefore, the vibrating apparatus was left in the water for extended periods and fish that had performed a circling behavior were not extracted. Therefore, individual fish could have engaged in multiple circling episodes and be present in multiple video clips. Due to the inability to discriminate if single individuals were responsible for repetitive events in the data, a different statistical analysis was used that could account for this limitation; different pools and years were counted as independent observations, so Pachón and Tinaja have an N = 7 each and Sabinos an N = 3. The number of left-side turns versus right-side turns was tested by fitting generalized linear mixed-effects models by using the binomial distribution (the Beta-distribution test gave equivalents and corroborating P values). Molino and Calera cave populations each had an N = 1, so no statistical analysis was performed. Examples of the video clips that were used by Espinasa et al. (2021) for Pachón cave can be seen at https://www.youtube.com/watch?v=RAvuH2MM0z8, and for Tinaja cave at https://www.youtube.com/watch?v=C0Zz7g4RChk.

Fish attracted to the vibration device in the video clips from Espinasa et al. (2021) show two types of behavior; they can swim towards the device and then swim away, or they can be highly responsive, swimming in circles around the device. Two types of data gathering were performed to discriminate highly responsive fish from the overall response of the population. To measure the side preponderance of the highly responsive specimens, all individual fish were tracked by one of the authors of this paper using the VLC Media Player to see if they circled the vibrating device clockwise or counterclockwise. When a fish circled the vibrator device for at least 270 degrees around the

device, showing at all times the same side of the face, it was counted as the first turn around the device. If the individual fish continued circling the device two, three, or more times, the number of turns was counted subsequently from the initial starting point. When a fish changed its path, so the other side of the face was towards the device, the number of turns for that side of the face stopped, and counting was restarted.

To analyze the overall response of both highly responsive and less responsive fish, the videos were divided into 5-second clips, and the frozen image at the end of each clip was analyzed. The number of fish inside a 6.25 cm radius circle in the still image was counted, as in Espinasa et al. (2021), but this time dividing the number of individuals facing the apparatus with their right side or left side of the body. Fish analyzed with this system included both the highly responsive fish that circled the device and the less responsive that swam away after approaching the device.

Previous VAB studies conducted in the laboratory (Yoshizawa et al. 2010) have been performed under the illumination of infrared light (880 nm wavelength, BL1960-880 blacklight, Advanced Illumination). As described in Espinasa et al. (2021), we attempted to use the Night Vision feature of the DCR-SR42 Sony Digital camera to record behavior in the field. Regrettably, it is of insufficient intensity to effectively be used in the trying conditions of the field. Muddy banks or pools at the bottom of small pits often did not allow the camera to be positioned close enough for the night vision illumination. It was found that the night vision illumination barely penetrated underwater, and fish could not be accurately seen. Videos analyzed were the same as those used in Espinasa et al. (2021), where illumination with standard headlamps (Sten light) was used to record when the vibrator was off and when it was on. As discussed in Espinasa et al. (2021), since the light was used both when the stimulus was absent and present and for fish using their left side and right side, it is not an experimental variable. The eyeless fish handedness response to the vibration stimulus was determined for both sides under identical illumination conditions.

Results

Our first hypothesis was that the curved body shape of *Astyanax* fish while turning around a vibrating stimulus, or the bent shape of a cavefish skull, has a size that allows them to efficiently reflect water waves with a function akin to a parabola (Fig. 1A, B). When we tested through mathematical modeling for sound (pressure waves) that travel through water at a speed close to 1,480 m/s. Results show that the corresponding theoretical wavelengths for 30 and 40 Hz used for VAB are 50 and 37 m (Fig. 1C). It is very unlikely that a small fish body (~ 10 cm) can work as a parabolic amplifier for sound at those wavelengths.

Yet, ripple waves are not pressure waves, and their mechanical manifestation is mainly by water flow, where fluid particles are in an orbital motion. This motion diminishes strongly with depth, so ripple waves stay very close to the surface, and have much slower speeds (Feynman et al. 1963). When modeling wave frequencies of 30–40 Hz

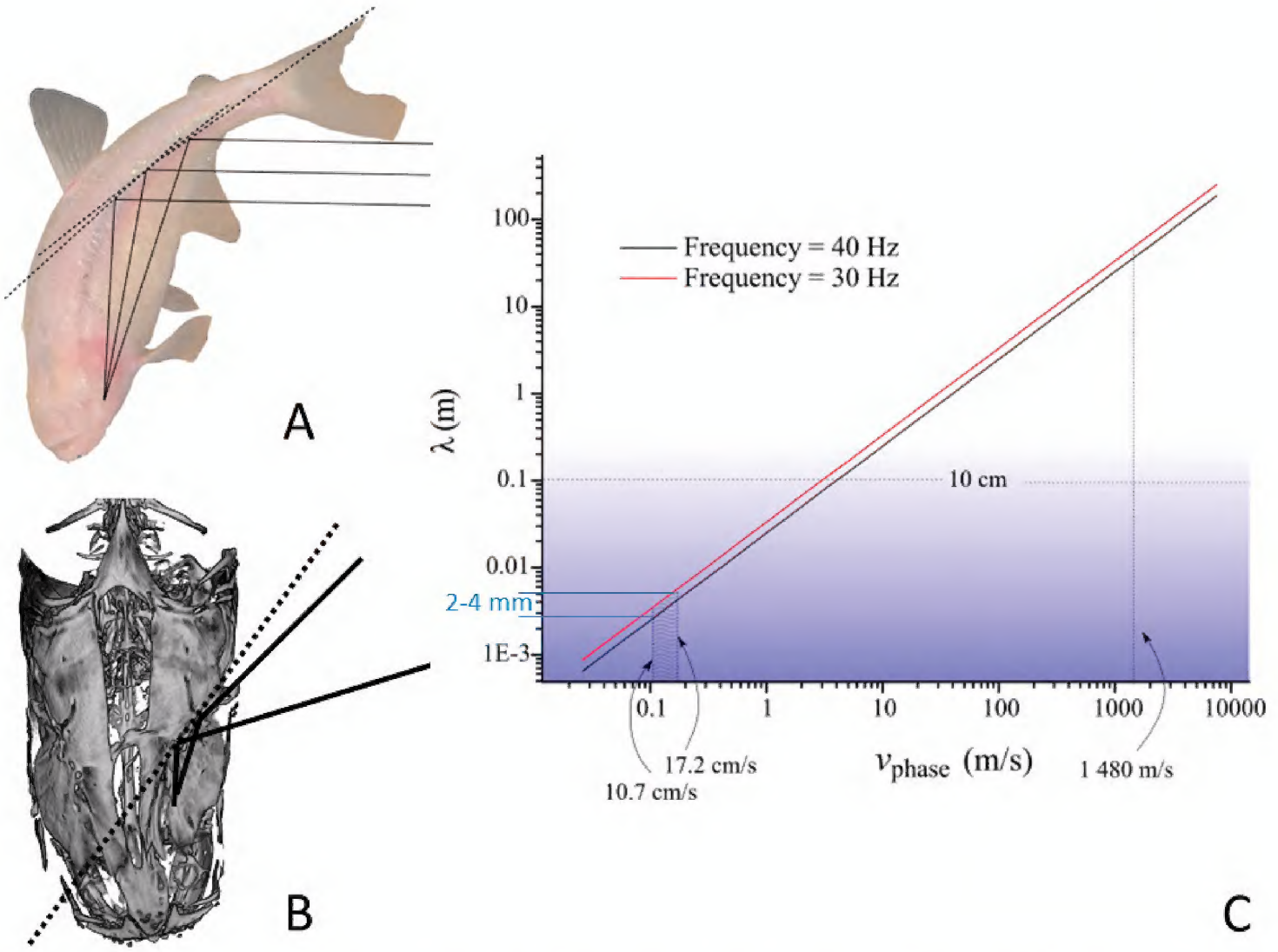

Figure 1. A schematic depiction of a plane wave (rays) reflected on the fish's body. In this case, the waves are being amplified towards the head neuromasts. The black dotted lines represent tangents to the fish lateral line at the reflection points **B** deflection of plane waves on the bent skull of a cavefish **C** wavelength is plotted against every phase velocity between 2 cm/s and 10,000 m/s (logarithmic scale). The blue shade aims to highlight the wavelength region for which parabolic amplifiers can work in a 10 cm long fish. Clearly, the speed of sound (1,480 m/s) is off limits for frequencies at around 35 Hz, but surface ripples that travel at 10.7–17.2 cm/s are within the proper wavelength range to be efficiently reflected by structures of a minimum length of 0.2–0.4 cm. Thus, curved bodies of a fish when circling around a vibrating stimulus or a bent skull can theoretically serve as amplifiers for the ripple waves generated by prey, but not for the sound that prey also produce. Skull images modified from Gross et al. (2014).

and a speed of 10.7–17.2 cm/s, which is the actual speed of ripples on the surface water of the pools inhabited by the cavefish as determined by Espinasa et al. (2021) results from mathematical modeling (Fig. 1C) showed that the theoretical minimum size for a surface to efficiently reflect this type of waves is between 2–4 mm. Thus, the length of an adult *Astyanax* and the size of their bent skulls are within the theoretical ranges to efficiently deflect this type of water waves towards the main superficial neuromasts responsible for mediating VAB.

Our second hypothesis was whether there is laterality in response to vibrating stimulus in their natural habitat. In the field, cavefish responded to the vibrations generated by approaching the device using the neuromasts of the left side of their face or their right side. Highly responsive individuals then circled the device counterclockwise

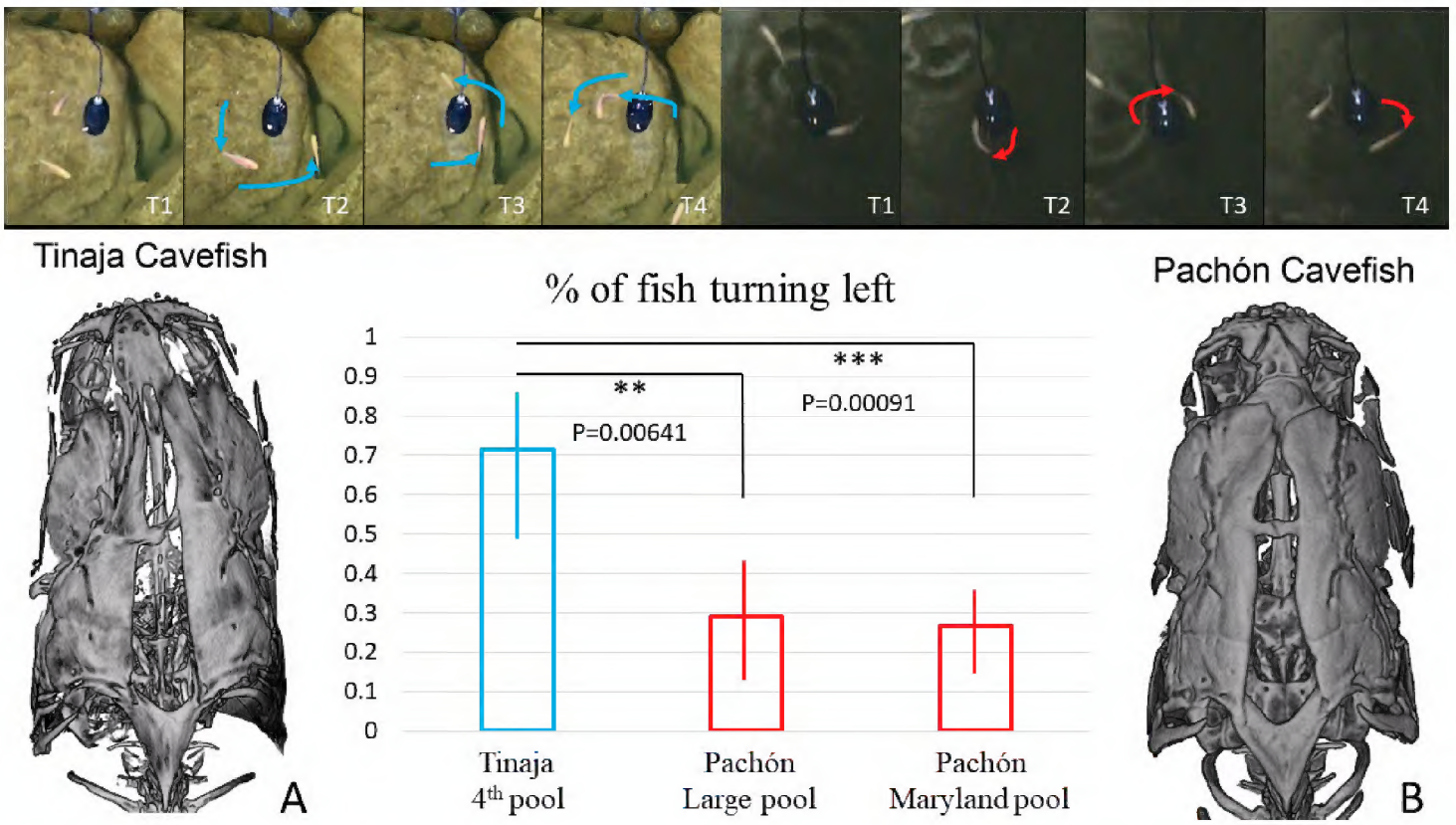

Figure 2. Cavefish explore a vibrating apparatus by circling counterclockwise (T1–T4 left), using their left side, or clockwise (T1–T2 right), using their right side. T1–T4 are sequential images from a video clip with arrows following the path of single fish while circling. Graph shows that preponderant side with which cavefish circle around a vibrating stimulus is significantly different between cave populations (Brackets for 90% confidence) **A, B** skull images modified from Gross et al. (2014) showing that the left bending of the skull is different between Tinaja and Pachón cavefish.

(Fig. 2 T1–T4 left) or clockwise (Fig. 2 T1–T4 right). When the device was off, all populations showed no right side or left side preponderance (all populations $P > 0.35$). When the device was vibrating, data shows that cave populations have a laterality or preponderant side with which to explore the source of vibrations (Figs 2, 3), But this laterality can be different among different caves: at Tinaja's 4th pool preponderance was for the left side (15 Left; 6 Right), while at Pachón's large pool (9 Left; 22 Right) and at Maryland's pool (15 Left; 41 Right) the preponderance was for the right side, as seen in figure 2 (Tinaja vs. Pachon's large pool $P = 0.00641$; Tinaja vs. Pachon's Maryland pool $P = 0.00091$). When analyzing only the highly responsive individuals that engaged in circling behavior, most populations showed a higher proportion of left turns (Figs 3, 4). In the Molino cave population, 80% (16 left vs. 4 right) of the turns were in a left direction. In Sabinos, 94.4% (34 left vs. 2 right; $P = 0.00096$). In Tinaja, 73.0% (134 left vs. 50 right; $P = 0.00675$). In Calera, 72.4% (119 left vs. 44 right). No P values are offered for Molino and Calera since their $N = 1$. Only at Pachón cave did the fish show a higher number on for the opposite right turns (36.6%; 100 left vs. 173 right; $P = 0.06051$).

The border-line of a $P = 0.06051$ from the Pachón population for not significantly differing from a 1:1 ratio warranted further analyses. In 2022, the data collection method was improved to eliminate the effect of individual fish performing multiple circling and their presence in multiple video clips. With this improved method, at

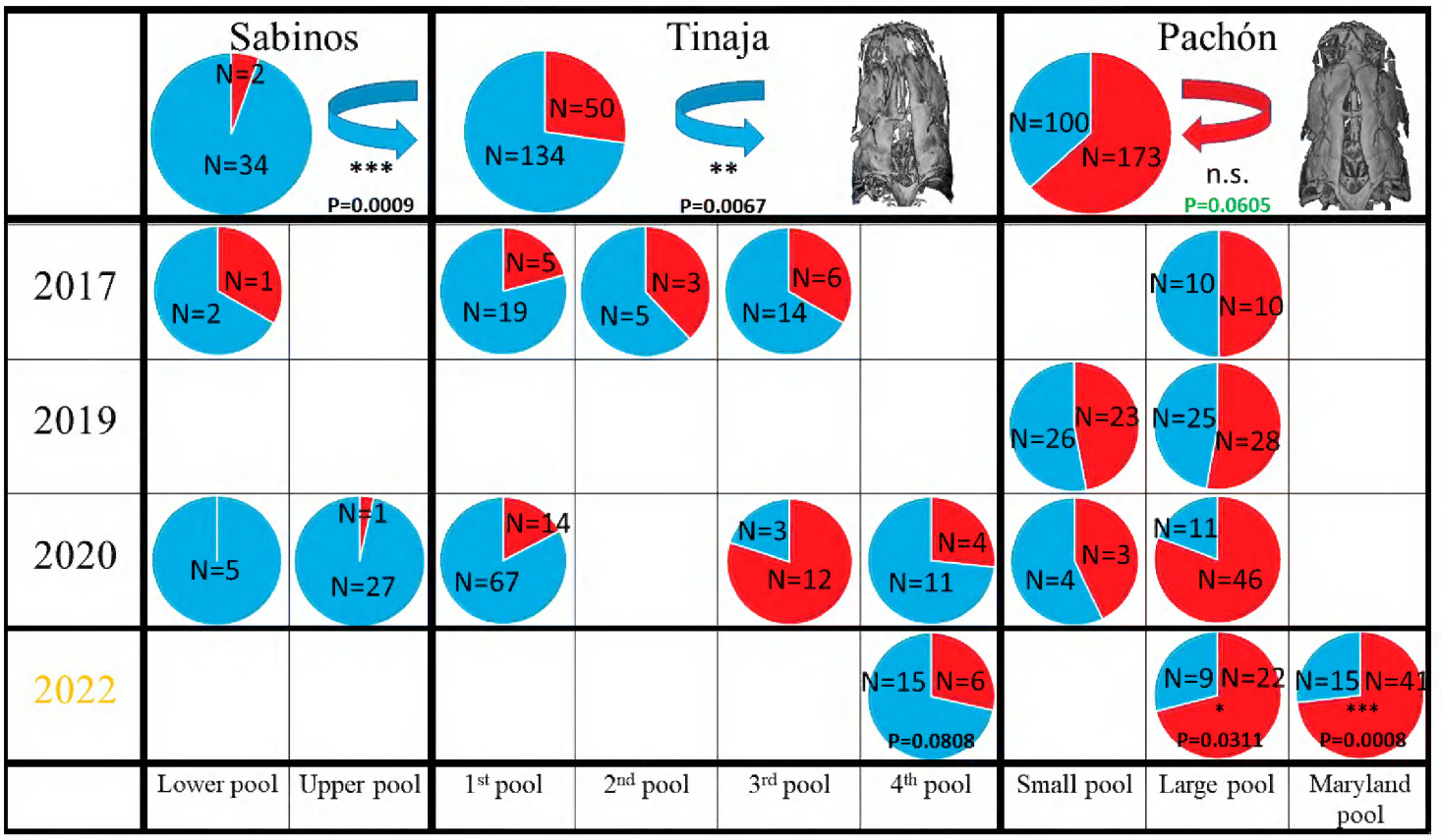

Figure 3. Preponderance in circling a vibrating stimuli using the left side or right side in different cave pools and times. As a population and throughout time, Sabinos and Tinaja cavefish used significantly more their left side. When all years and pools are included, Pachón showed a border line P = 0.0605 (in green) for not significantly differing from a 1:1 ratio. N's in upper row are for all four years. But when the enhanced data collecting method was used in 2022, fish from two Pachón pools significantly used their right side more. Videos from 2017–2020 are from Espinasa et al. (2021). For the recordings of 2022, methodology was improved such that it guaranteed that data was from independent fish within the population, allowing for statistical analyses for that single pool and date.

Pachón's Maryland pool, only 26.7% (15 left vs. 41 right; P = 0.00083) of the fish that circled were in a left direction, and in the large pool, 29.0% (9 left vs. 22 right; P = 0.03114).

For some caves, the preponderant side preference was relatively high among pools and in different years (Fig. 3). It is known that both Sabinos and Tinaja caves are part of a single hydrologic system (Elliott 2018), where cavefish can probably move from one cave to the other in extreme rainy seasons. Out of ten measurements in these two caves from four years of observations in a total of six different pools, only once did left-preponderance not dominate in their response to VAB. But it appears that side preponderance can also change over time and among pools. For Pachón cave (Fig. 3), four data points hovered around a 1:1 ratio, but in three, there was a preponderance for the right side (As mentioned above, two of them were significantly different from a 1:1 ratio).

The above results were for only highly responsive fish that circled the vibrating apparatus. As described in the methods, we did a second type of analysis that includes less responsive fish that just approached the device and then swam away, plus the responsive fish that circled the device. In this case, an intriguing trend was found for all populations: highly responsive fish appear to proportionally use their left side more

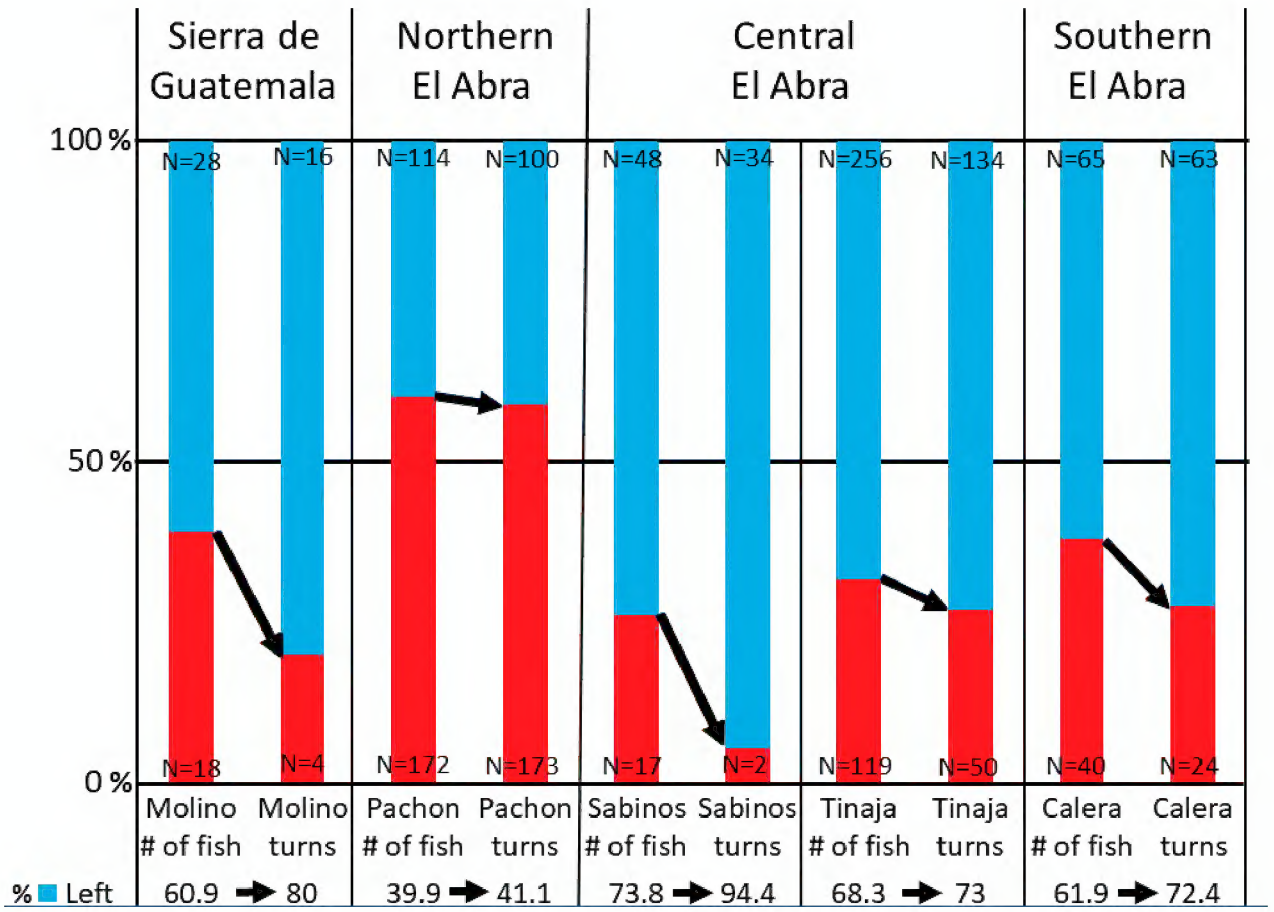

Figure 4. Highly responsive fish tend to proportionally use their left side more when compared to those individuals that display a less responsive behavior towards a vibrating stimulus. "# of fish" includes both individuals that only approach the device and then swim away and highly responsive fish that circle around the device. "Turns" counts the behavior exclusively of the highly responsive fish that circle the device. It would appear that highly motivated fish enhance their left-sidedness in their foraging behavior when responding with VAB, except perhaps in Pachón cave population.

when compared to less responsive individuals (Fig. 4). At Molino cave, right side use decreased from 39.1% to 20% when comparing data that includes less responsive fish that approach and then leave without circling versus only fish that circle. Similar decrease of right-side use was seen in Sabinos (26.2% to 5.6%), Tinaja (31.7% to 27.0%), Calera (38.1% to 27.6%). In other words, highly responsive fish seem to increase their left side preference. Only in Pachón cave did there appear to be no to minimal change (60.1% to 58.9%).

Discussion

Our results show that the head and body of *Astyanax* cavefish are within the theoretical size range to serve a function akin to a parabola for ripple waves being amplified for VAB, but not for sound (Fig. 1). Potential prey makes both sound waves and ripple waves. Both signals may be used by cavefish. What we have shown is that if there is an adaptive reason for the bent skull, it is unlikely that it is for amplifying sound waves, but it is at least theoretically possible that it is for amplifying ripple waves. Future

studies may resolve if ripple waves are actually being deflected in such a way that it amplifies the signal and if the bent skull shape of cavefish is implicated.

An alternative for the origin of a bent skull is that what is being selected is the neuromast number or properties, and suborbital bones are just developmentally linked. Powers et al. (2018) have shown that the canal neuromast position prefigures developmental patterning of the suborbital bone series in *A. mexicanus* fish. It may be that there is a selective advantage to asymmetric neuromast and the skull gets bent through pleiotropy or linkage.

Apart from a bent skull predominantly to the left (Powers et al. 2017), previous studies have shown that there is a correlation of VAB with left side number of neuromasts in Pachón cave population (Fernandes et al. 2018), and a QTL signal for the right-side number of neuromasts (Powers et al. 2020). These literalities suggest the possibility of swimming side preference for different behaviors to enhance sensitivity depending on the types of stimuli. It is known that cavefish prefer to use their right-side lateral line when encountering a novel landmark (De Perera and Braithwaite 2005), but before this study, it was unclear if there was a side preponderance for any foraging behavior including VAB.

We tested for laterality in response to VAB in the field and our data supports that all cave populations expressed laterality and significantly differed from an expected 1:1 ratio on the side with which they circle a potential prey, at least in some years and for some pools (Fig. 3). *Astyanax* populations from four out of the five caves studied (Molino, Sabinos, Tinaja, and Toro system) preferentially circled with their left side of the face towards the source of vibrations. In some years, subpopulations in several of Pachón's cave pools had no statistically significant lateral preponderance, but at other times they showed a significant preponderance for using their right side towards the stimulus (Figs 2, 3). It remains to be tested if VAB differences in preference of side between these cave populations are due to environmental conditions, skull differences, neuromast differences, or other genetic reasons. One hypothesis could be that in evolving for foraging in the darkness, laterality is enhanced through asymmetric sensitivity for different modes of stimuli or lateral swimming preference. Yet, the side itself may be irrelevant. It may well be that evolutionary histories, adaptive constraints, or stochastic circumstances allowed Pachón's population to have percentages of individuals that have left-preference or right-preference different from other caves and perhaps variable at different times.

Espinasa et al. (2021) showed that VAB expression could be variable within a single cave and among pools and found it to be correlated to environmental variables such as the amount and type of available food. It would appear that individual fish may change their expression of VAB according to their physiology, motivation, and nutritional state. Our results suggest that the proportional use of sidedness is also modified depending on the responsiveness of individual fish. It may be that fish that are hungry and more motivated to search for food rely differently on specialized sensory mechanisms affecting sidedness. One could expect differences between pools where most food consists of guano and pools with mostly vibrating prey. Support for this notion can be found by comparing subpopulations in different pools at Pachón cave. Espinasa

et al. (2021) noticed that Pachón's large pool contained low levels of organic carbon while the small pool, which has a small bat roost on top, had a significantly higher level of organic carbon. Our results show that both in 2019 and 2020, the large pool, which presumably has a lower density of guano-derived nutrients, had proportionally higher laterality favoring the right side than the smaller pool, which has higher organic densities (See fig. 3; 2019 = 53.0% left in the small pool vs. 47.1% left in the large pool; 2020 = 57.1% left in the small pool vs. 19.2% left in the large pool).

Caution should be used when evaluating results in this and future studies since the preponderance for laterality during foraging behavior may fluctuate due to environmental conditions and over long periods of time. The studies by Gross et al. (2015) and Powers et al. (2017) have shown that in laboratory-bred cavefish, there is variability in how bent the skull is. While the majority have a left bent in both Pachón- and Tinaja-derived fish, some individuals can have a right-bent skull. A limitation of behavioral studies with laboratory stock-fish is that they are derived from a few field-collected specimens that have been bred for several generations, and some bottleneck effects can be expected. Laboratory animals could be expressing a preponderance in side of their behavior different from the one currently being expressed in the field. For example, Espinasa et al. (2021) showed that while some laboratory stock populations derived from Tinaja and Molino were reported as VAB reduced (Yoshizawa et al. 2010, 2015; Yoshizawa 2016), in the field, those same caves have abundant VAB positive individuals. Likewise, Pachón laboratory stock animals are derived from field collections done before 2017. In our data, Pachón population did not show clear laterality in 2017 and 2019, but after 2020 subpopulations had significant VAB laterality.

In conclusion, while previous studies have shown that cavefish from Pachón cave prefer to use their right-side lateral line when encountering a novel stationary landmark (De Perera and Braithwaite 2005), this is the first time that it has been shown that *Astyanax* cavefish show laterality in their foraging behavior in the field. Likewise, we have shown that a cavefish's skull is of a size that at least theoretically could serve as an amplifier, thus allowing for its bent shape to have an adaptive function.

Conclusions

1) If VAB mediating neuromasts are more proficient on one side of the head, our theoretical analyses have shown that the size of a bent skull or of a whole-body flexed when circling a vibrating stimulus is sufficient to provide an efficient surface on which to reflect and concentrate wave signals towards that spot (Fig. 1).

2) *Astyanax* cavefish show laterality in the preponderant side they use to explore vibrating stimuli (Fig. 3).

3) Which side is preponderant can vary between different cave populations (Fig. 2).

4) Laterality towards VAB may be plastic; how responsive or motivated a particular individual is to respond towards a vibrating stimulus can have an effect on which side they preferentially use (Fig. 4).

5) We have found some degree of variability in the expression of laterality among pools, and throughout time within a single cave. The proportion of individuals within a population that have a certain lateral number of sensory neuromasts, bending of their skulls, and/or any other characters that may be involved in lateralization of foraging behavior may change due to stochastic effects (bottlenecks) and/or natural selection due to local environmental conditions.

6) Caution should be used when comparing VAB and laterality results. Nutrition levels in the different pools in the field and the laboratory may be drastically different. Likewise, acclimatization to preferentially eat live prey, guano, or even pet food throughout their development may accustom individuals to use different types of receptors.

Significance statement

Vibration attraction behavior (VAB) confers the ability to swim toward water disturbances generated by prey in the darkness. VAB has a genetic component and evolved in cavefish. Cavefish also have a unique asymmetry of a "bend" in their skulls and a correlation of VAB only with the left side number of neuromasts. An asymmetric body pattern may negatively affect swimming performance and create an unequal lateral hydrodynamic resistance (drag). A possible explanation is that as an adaptive compensation, lateralization may enhance the dynamics of water signaling, signal reception, or signal processing on one side. Our results support this association in showing there is laterality in VAB's response, where a vibrating object is examined preferentially with one side of the face. However, which laterality, right side or left side, appears to be variable among different cave populations. Perhaps the adaptative nature of this phenomenon selects for asymmetry by itself and does not necessarily select for which side is the one to be specialized.

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